Amendments to the Specification

Please replace the title with the following title:

--A METHOD OF PRESERVING TISSUE.--

Please replace paragraphs **[001]-[009]** at pages 1-3, with the following paragraphs:

--~~[001]~~ **[0001]** This application claims the benefit of U.S. Provisional Application No. 60/432,957, filed December 11, 2002, which is incorporated by reference in its entirety.

BACKGROUND

~~[002]~~ **[0002]** This invention relates to the sterilization and cryopreservation of tissues for storage. The tissues may be harvested from human or animal subjects and are then processed and cryopreserved (frozen) for later implantation. Allograft tissues, including, but not limited to, heart valves and portions of heart valves, aortic roots, aortic walls, connective tissues including fascia and dura, vascular grafts (including arterial, venous, and biological tubes), and orthopedic soft tissues, such as boned- or non-boned tendons or ligaments, are often subjected to cryogenic preservation. In this manner, a ready supply of these valuable tissues can be made available for later implantation into mammals, especially humans. In addition, viable xenograft tissues from transgenic animals or tissues developed from human or non-human cells that may include differentiated cell types, stem cells, or genetically-modified cells of various origins may be appropriately processed, cryopreserved, and stored for later implantation.

~~[003]~~ [0003] In addition to living allograft, xenograft, or bio-engineered tissues, which may be cryopreserved, living tissues may be decellularized to render them acellular. While living tissues are often decellularized before cryopreservation, they can also be decellularized after cryopreservation and storage.

~~[004]~~ [0004] Generally, decellularization involves substantially reducing the living, non-structural constituents within the tissue. This may be achieved by first exposing the tissue to a hypotonic solution to lyse the cells and then subjecting the tissue to a nuclease treatment to degrade nucleic acids. A more detailed discussion of tissue decellularization may be found in U.S. Pub. No. US 2001/0000804 A1, which is incorporated by reference in its entirety, except that in the event of any inconsistent disclosure or definition from the present application, the disclosure or definition herein shall prevail.

~~[005]~~ [0005] Cryopreservation may be a preferred method of preserving living or decellularized tissue for extended storage. By cryopreserving a tissue in a suitable cryoprotectant, it may be possible to reduce the damage to the tissue that can occur during uncontrolled rate freezing, freeze-drying, and glutaraldehyde preservation methods. In contrast to uncontrolled rate freezing and freeze-drying, cryopreservation preferably involves the addition of one or more cryoprotectant containing solution to the tissue followed by a slower, controlled-rate freezing regimen, which can be adjusted to the particular requirements of each tissue to which it is applied. Cryoprotectants can limit cell or tissue damage due to the formation of ice (water) crystals during freezing and thawing.

~~[006]~~ [0006] In general, there are two requirements for the successful cryopreservation of living tissue. First, the harvested tissue should be frozen to a sufficiently low temperature so metabolic activity effectively ceases within the cell, without destroying the cell. Second, the cryopreservation and thawing regimens should have minimal effects on tissue cell viability and limit structural damage to the tissue. When decellularized tissue is cryopreserved, the primary focus is preventing damage to the structure of the extracellular matrix.

~~[007]~~ [0007] Varying cryoprotectant containing solutions have been used to cryogenically preserve biological tissues for later implantation. Some cryopreservation solutions use a combination of *dim*ethylsulf*o*xide (DMSO) and *F*etal *B*ovine *S*erum (FBS) with other constituents for tissue preservation. In these solutions, DMSO may provide interference with the ability of water to form ice crystals during freezing and radical scavenging. However, a disadvantage of DMSO based cryopreservation solutions is the unpleasant odor given off from irradiated solutions upon thawing. DMSO containing cryopreservation solutions can also demonstrate toxicity to living tissues above 4° C. This toxicity and the detailed thawing procedures required to limit its adverse affects on heart valves are described in U.S. Pat. No. 4,890,457, for example.

~~[008]~~ [0008] Because Fetal Bovine Serum is animal-derived, its use in cryopreservation solutions may introduce undesirable contaminants. In addition to the risk of contamination by bacteria and viruses, prion contamination is also possible. It is currently believed that Creutzfeldt-Jakob disease or variant bovine spongiform encephalopathy is transmitted through prions. Not only does FBS open the possibility of disease transmission, but due to

the limited availability of cows believed free of prion transmitted disease, FBS can only be harvested from a limited number of herds in specific countries.

~~[009]~~ [0009] Unlike tissues sourced from other human individuals (allografts), which are classified by regulatory authorities as tissues intended for transplantation and whose processing includes procedures to reduce the risk of contamination by pathogenic agents, tissues sourced from different species (xenografts) are classified as medical devices and must be terminally sterilized prior to implantation. Sterilization techniques and conditions are preferably selected to provide a high level of assurance against contamination of the tissue with microbes, while limiting damage to the structure and function of the tissue. These same techniques may also reduce the activity or infectivity of other pathogenic agents, such as viruses, thus increasing the safety factor of the implantable device.--

Please replace the paragraph **[0033]**, starting at page 9 and continuing at page 10, with the following paragraph:

--**[0033]** Biological tissues, which may be preserved in the claimed solutions, include any tissue that is appropriate for implantation into humans or animals. The implantable tissue can be human or non-human, such as bovine, porcine, or non-human primate, in origin. Suitable tissues include, but are not limited to, partial organs, blood cells, blood proteins, heart valve leaflets, heart valves, aortic roots, aortic walls, pulmonary valves, pulmonary conduits, non-valved conduits, mitral valves, monocusps, tendons, ligaments, ~~facia~~ fascia, large and small vascular conduits, blood vessels, arteries, veins, diaphragm, pericardium, umbilical cords, and dura ~~matter~~ mater or tympanic membranes. Especially

preferred tissues suitable for cryopreservation in the claimed solutions are collagen-rich tissues, such as heart valves, vessels or conduits suitably applicable to blood vessel replacement or repair, and tendons.--

Please replace the paragraph **[0053]** with the following paragraph:

--**[0053]** Preferable radical scavengers for use in the present invention are acids and salts that ionize in water. Preferable radical scavengers include, but are not limited to, sodium ascorbate, carotenoids, 1-ascorbic acid, d-isoascorbic acid, sodium sulfite, sodium metabisulfite, sulfur dioxide, nicotinic acid, nicotinic acid amine, cysteine, glutathione, sodium nitrate, sodium nitrite, ~~flavenoids~~ flavonoids, selenium, alpha-lipoic acids, acetyl cysteine, water-soluble tocopherol derivatives including sodium Vitamin E phosphate (VEP), lauryl imino dipropionic acid tocopheryl phosphate, tocopheryl glucoside, tocopheryl succinate, Tocophersolan (tocopheryl polyethylene glycol 1000 succinate), Tocophereth-5,10,12,18, and 50 (polyethylene glycol (PEG) tocopheryl ethers), Lazaroids, ubiquinone (coenzyme Q_{10}) butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), analogs thereof, isomers thereof, derivatives thereof, and mixtures thereof. More preferred radical scavengers include sodium ascorbate, water-soluble derivatives of ascorbate, carotenoids, and mixtures thereof. At present, an especially preferred radical scavenger for use in the claimed solutions is sodium ascorbate.--

Please replace the paragraph **[0063]** with the following paragraph:

--**[0063]** The cryopreserved tissue is preferably packaged prior to freezing and irradiation. Packaging may occur before or after freezing. Suitable packaging materials include, but are not limited to, high-density polyethylene, foil/polyolefin, foil/polyester, and laminates, such as, ~~polyolefin/nylon/Mylar®~~ polyolefin/nylon/polyester film (MYLAR®). Preferably, the package

is in the form of one or more envelopes, which may be evacuated prior to closure.--

Please replace the paragraph **[0075]** with the following paragraph:

--**[0075]** A solution in accordance with the present invention was made by combining the following constituents in sterile, deionized water:
sterile phosphate buffered saline (Biowhitakker, #17-516Q),
0.154 M NaCl,
12.5% (w/v) polyvinylpyrrolidone (PVP, ~~Kollidone®~~ polyvinylpyrrolidone polymer (KOLLIDONE®) 17 PF, BASF Corporation, General Office, (318) 861-8201, 8800 Line Ave, Shreveport, LA 71106),
15% (v/v) isopropanol, and
0.5 M sodium ascorbate. Molarities are for the total cryopreservation solution.--